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PATENT COOPERATION TREATY

JSvn

by fax and post

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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ABEL & IMRAY
CASE NO. 6290
G.O.
10 DEC 2001
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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	07.12.2001
Applicant's or agent's file reference: JSvn/6290	IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06533	10/07/2000	09/07/1999

Applicant: THE EUROPEAN MOLECULAR BIOLOGY LABORATORY et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Büchler, S Tel.+49 89 2399-8090

Form PCT/IPEA/416 (July 1992)

PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

14

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
JSvn/6290		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP00/06533	10/07/2000	09/07/1999
International Patent Classification (IPC) or national classification and IPC		
C12N15/10		
Applicant		
THE EUROPEAN MOLECULAR BIOLOGY LABORATORY et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 13 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
06/02/2001	07.12.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Chakravarty, A Telephone No. +49 89 2399 8536

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06533

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:
Description, pages:

1-71	as originally filed	

Claims, No.:

1-48	with telefax of	29/10/2001

Drawings, sheets:

1/13-13/13	as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   - ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
   - ☐ the language of publication of the international application (under Rule 48.3(b)).
   - ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   - ☐ contained in the international application in written form.
   - ☐ filed together with the international application in computer readable form.
   - ☐ furnished subsequently to this Authority in written form.
   - ☐ furnished subsequently to this Authority in computer readable form.
   - ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
   - ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   - ☐ the description, pages:
   - ☐ the claims, Nos.:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP00/06533

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,12-18
	No:	Claims	1,3-11,19-48
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-48
Industrial applicability (IA)	Yes:	Claims	1-48
	No:	Claims	

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
see separate sheet

INTERNATIONAL PRELIMINARY EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/EP00/06533

Re Item I

Basis of the report

The amendments filed with the International Bureau under Article 19(1) introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 19(2) PCT. In particular, it is doubtful whether the passages indicated by the applicant actually provide basis for the amendments.

Re Item V

Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

The present application is concerned with method for introducing a double stranded target DNA into a vector by means of homologous recombination. The aim of the invention is to provide high-efficiency targeted cloning and sub-cloning. This is done with the aid of bacterial recombinases, e.g. RecE/T or Redα/β.

Reference is made to the following document:

D1: WO-A-99/29837

Novelty and inventive step.

D1, which comes from the same applicants, is directed to a method for cloning DNA molecules using a homologous recombination mechanism.
D1 and the present application appear to address largely identical subject-matter differing mainly in the formulation of the claims.

Applicant submits that the present application differs from D1 in that the present invention a vector is constructed that contains synthetic sequences called "homology arms," specifically designed to capture a chosen target DNA sequence between its arms by targeted homologous recombination. These synthetic sequences contain sequence homology to the termini flanking the target DNA sequence, providing substrates for targeted homologous recombination, which results in the capture of the target DNA into the vector homology arms, resulting in a cloned DNA molecule.
The present invention is distinguished from previous homologous recombination cloning methods such as those described in DI. In fact, D1 took the opposite approach,

i.e., sequences on a targeting DNA molecule, preferably a linear DNA molecule, were designed and constructed to be homologous to sequences on a preexisting target vector DNA.
Moreover, while the methods disclosed in DI are useful for inserting a DNA sequence of choice into a preexisting vector, D1 does not describe methods for the cloning of a target DNA sequence from a complex mixture of DNA molecules, for example, a genomic DNA library, without having to resort to conventional cloning methods. In order to use such methods to done such a sequence from a genomic library, one would first have to clone and isolate the chosen target DNA molecule, introduce regions of homology at its ends, and then recombine it into the cloning vector. In contrast, with the methods of the present invention one may directly clone a target DNA sequence of choice without first having to clone and isolate the chosen target DNA molecule, eliminating time consuming and labour-intensive cloning steps.

While the IPEA notes from the present description that the applicants may well have achieved the above advantages over, D1 but these are not properly reflected in the claims, which still lack novelty over D1. For claim 1 and related claims : the indication "designed to be" carries no technical content whatsoever and leaves it open that subject-matter of D1 and the present subject-matter are identical.

Claim 2 and dependent claims appear to be novel because D1 does not disclose a methods where a vector is introduced into a cell that already contains a target DNA.

However, none of the novel subject-matter can be regarded as involving an inventive step. The fact that in claim 2 the cell already contains the target DNA do not appear to be associated with any particular surprising effect.

Claim 19 lacks novelty because it reads on any vector containing an origin of replication. This is because the "homology arms" are defined by an unspecified "target", which could be any sequence.
This objection applies *mutatis mutandis* to claims 20-24.

Re Item VII

Certain defects in the international application

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art

disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

Re Item VIII

Certain observations on the international application

1 The following terms are not clear: terminus, homologous (is identity required? If so over what length?). The applicant should amend the claims by introducing the definitions of the preceding terms from the description, page 31).

2 It is clear from the description (see page numbers cited below), that the following feature is essential to the definition of the invention:
page 31, line 29: the orientation of the two arms relative to the insert must be the same as the orientation of the homologous sequence relative to the target DNA.

Since independent claims do not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

2.1 The claims make no mention of the cloning from libraries, which appears to be an essential feature. Also, study of the figures shows that the vector used should be linear, with a gap between the homology arms. This feature too is lacking from the claims.

3 The description of the component order in the vector is not clear because it is not specified whether the components are inside or outside the homology arms.

4 Although claims 1-7, have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought ..and/or.. in respect of the terminology used for the features of that subject-matter. The aforementioned claims therefore lack conciseness. Moreover, lack of clarity of the claims as a whole arises, since the plurality of independent claims makes it difficult, if not impossible, to determine the matter for which protection is sought, and places an undue burden on others seeking to establish the extent of

the protection.

Hence, claims 1-7 do not meet the requirements of Article 6 PCT.

WHAT IS CLAIMED IS:

1. A method for introducing a double-stranded target DNA into a vector comprising culturing a bacterial cell that expresses a functional recombinase, said bacterial cell containing (a) the target DNA comprising a first double-stranded terminus and a second double-stranded terminus, and (b) a vector DNA comprising, in the following order along the vector DNA strand: (i) a first double-stranded homology arm; (ii) an origin of replication; and (iii) a second double-stranded homology arm, such that the sequence of a vector DNA strand of the first homology arm is designed to be homologous to the sequence of a target DNA strand of the first terminus, and the sequence of a vector DNA strand of the second homology arm is designed to be homologous to the sequence of the target DNA strand of the second terminus, such that the target DNA is inserted into the vector DNA between the homology arms.

2. A method for making a cell containing a recombinant DNA molecule, said method comprising:

a) introducing a first and a second strand of a double-stranded vector into a cell, said cell containing a double-stranded target DNA and expressing a bacterial recombinase, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along said first vector DNA strand: a first homology arm, one strand of the origin of replication, and a second homology arm;
said target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, the target DNA sequence, and a second terminus,
such that the sequence of the first homology arm on said vector DNA strand is homologous to the sequence of the first terminus on said target DNA strand, and the sequence of the second homology arm on said vector DNA strand is homologous to the sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

3. A method for making a cell containing a recombinant DNA molecule, said method comprising:

introducing a first and a second strand of of a double-stranded vector and first and second double-stranded adaptor oligonucleotides into a cell, said cell containing a double-stranded target DNA and expressing a bacterial recombinase,

said vector comprising an origin of replication and two double-stranded homology arms, in the following order from 5' to 3' along said first vector DNA strand: a first homology arm, the origin of replication, and a second homology arm;

said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, a target DNA sequence, and a second terminus;

said first oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is designed to be homologous to the nucleotide sequence of the first terminus on said target DNA strand;

said second oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is designed to be homologous to the nucleotide sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

4. A method for making a cell containing a recombinant DNA molecule, said method comprising:

a) introducing a first and a second strand of a double-stranded target DNA molecule into a cell, said cell containing a vector and expressing a bacterial recombinase,
said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along said first target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm;
such that the sequence of the first homology arm on said vector DNA strand is designed to be homologous to the sequence of the first terminus on said first target DNA strand, and the sequence of the second homology arm on said vector DNA strand is designed to be homologous to the sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

5. A method for making a cell containing a recombinant DNA molecule, said method comprising:

a) introducing a first and a second strand of a double-stranded target DNA molecule and a first and second double-stranded oligonucleotide into a cell, said cell containing a vector and expressing a bacterial recombinase,
said target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along said first target DNA strand: a first terminus, a target DNA sequence, and a second terminus;
said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm;
said first double-stranded oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first

nucleotide sequence and a second nucleotide sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence designed to be homologous to the nucleotide sequence of the first terminus on said target DNA strand;

said second double-stranded oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is designed to be homologous to the nucleotide sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

6. A method for making a cell containing a recombinant DNA molecule, said method comprising:

a) introducing a first and a second strand of a double-stranded vector and a first and a second strand of a double-stranded target DNA into a cell expressing a bacterial recombinase,

said double-stranded vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along said first vector DNA strand: a first homology arm, the origin of replication and a second homology arm,

said double-stranded target DNA comprising a target DNA sequence and two termini, in the following order, from 3' to 5' along said first target DNA strand: a first terminus, a target DNA sequence; and a second terminus;

such that the nucleotide sequence of the first homology arm on said vector DNA strand is designed to be homologous to the nucleotide sequence of the first terminus on said target DNA strand, and the nucleotide sequence of the second homology arm on said vector DNA strand is designed to be

homologous to the sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

7. A method for making a cell containing a recombinant DNA molecule, said method comprising:

a) introducing a first and a second strand of a double-stranded vector, a first and a second strand of a double-stranded target DNA molecule, and a first and second double-stranded adaptor oligonucleotide into a cell expressing a bacterial recombinase,

said vector comprising an origin of replication and two double-stranded homology arms, in the following order from 5' to 3' along said first vector DNA strand: a first homology arm, the origin of replication and a second homology arm;

said target DNA comprising a target DNA sequence and two double-stranded termini, in the following order, from 3' to 5' along a target DNA strand: a first terminus, the target DNA sequence, and a second terminus;

said first oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is designed to be homologous to the sequence of the first terminus on said target DNA strand;

said second oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5', a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is designed to be homologous to the nucleotide sequence of the second terminus on said target DNA strand; and

b) subjecting the cell to conditions that allow intracellular homologous recombination to occur.

8. The method of claim 6 wherein the cell further contains a nucleotide sequence encoding a site-specific recombinase operatively linked to a promoter, and the vector further comprises a first and second recognition site for the site-specific recombinase, a first recognition site located outside the first and second homology arms, and a second site-specific recombinase recognition site located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific recombinase.

9. The method of claim 7 wherein the cell further contains a nucleotide sequence encoding a site-specific recombinase operatively linked to a promoter, and the vector further comprises a first and second recognition site for the site-specific recombinase, a first recognition site located outside the first and second homology arms, and a second site-specific recombinase recognition site located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific recombinase.

10. The method of claim 6 wherein the cell further contains a nucleotide sequence encoding a site-specific endonuclease operatively linked to a promoter, and the vector further comprises a recognition site for the site-specific endonuclease located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific endonuclease.

11. The method of claim 7 wherein the cell further contains a nucleotide sequence encoding a site-specific endonuclease operatively linked to a promoter, and the vector further comprises a recognition site for the site-specific endonuclease located inside the first and second homology arms; and during or after step b), inducing expression of the site-specific endonuclease.

12. The method of any one of Claims 2-11 wherein the vector further comprises a selectable marker located outside the first and second homology arms, such that the vector comprises, in either of the following two orders from 5' to 3' along a vector DNA strand: i)

the first homology arm, the selectable marker, the origin of replication and the second homology arm, or ii) the first homology arm, the origin of replication, the selectable marker, and the second homology arm.

13. The method of Claim 12 wherein the selectable marker confers antibiotic resistance to the cell containing the vector.

14. The method of any one of Claims 2-11 wherein the bacterial recombinase is RecE/T or Redα/β recombinase or both RecE/T and Redα/β recombinases.

15. The method of any one of Claims 2-11 wherein the cell is a bacterial cell.

16. The method of any one of Claims 2-11 wherein the cell is an *E. coli* cell.

17. The method of any one of Claims 2-11 wherein the cell is a eukaryotic cell that recombinantly expresses RecE/T and/or Redα/β recombinase.

18. A method for making a recombinant DNA molecule comprising the method of any one of Claims 2-11 which further comprises isolating a recombinant DNA molecule that comprises the target DNA sequence inserted into the vector.

19. A double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; such that the nucleotide sequence of the first homology arm on a first vector DNA strand is homologous to the sequence of the first terminus on a first target DNA strand, and the nucleotide sequence of the second homology arm on the first vector DNA strand is homologous to the nucleotide sequence of the second terminus on the first target DNA strand.

20. The vector of Claim 19 wherein the origin of replication is a bacterial origin of replication.

21. The vector of Claim 19 wherein the origin of replication functions in *E. coli*.

22. The vector of Claim 19 wherein the origin of replication functions in a mammalian cell.

23. A cell comprising a double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; such that the nucleotide sequence of the first homology arm on a first vector DNA strand is designed to be homologous to the sequence of a first terminus on a first target DNA strand, and the nucleotide sequence of the second homology arm on the first vector DNA strand is designed to be homologous to the nucleotide sequence of a second terminus on the first target DNA strand.

24. The cell of Claim 23 which is a bacterial cell.

25. A kit useful for directed cloning or subcloning of a target DNA molecule comprising in one or more containers:

a) a double-stranded DNA vector useful for directed cloning or subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; such that the nucleotide sequence of the first homology arm on a first vector DNA strand is designed to be homologous to the sequence of a first terminus on a first target DNA strand, and the nucleotide sequence of the second homology arm on the first vector DNA strand is designed to be homologous to the nucleotide sequence of a second terminus on the first target DNA strand; and

b) a cell containing a bacterial recombinase.

26. The kit of Claim 25 wherein the homology arms have sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.

27. The kit of Claim 25 wherein the first and second double-stranded oligonucleotide have nucleotide sequence homology to a BAC, PAC, lambda, plasmid or YAC based cloning vector.

28. A kit useful for directed cloning or subcloning of a target DNA molecule comprising in one or more containers:

a) a double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm; and

b) a first double-stranded adaptor oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first sequence and a second sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is designed to be homologous to the nucleotide sequence of a first terminus on a target DNA strand;

c) a second double-stranded adaptor oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth nucleotide sequence is designed to be homologous to the nucleotide sequence of a second terminus on said target DNA strand; and

d) a cell containing a bacterial recombinase.

29. The kit of Claim 25 or 28 wherein the cell is an *E. coli* cell.

30. The kit of Claim 25 or 28 wherein the cell is a frozen cell competent for uptake of DNA.

31. A kit useful for directed cloning or subcloning of a target DNA molecule comprising in one or more containers:

a) a double-stranded DNA vector useful for directed cloning and subcloning of a target DNA molecule of interest, said vector comprising an origin of replication and two homology arms, in the following order from 5' to 3' along a vector DNA strand: a first homology arm, the origin of replication and a second homology arm;

b) a first double-stranded adaptor oligonucleotide comprising a first oligonucleotide DNA strand comprising, in the following order, from 3' to 5': a first nucleotide sequence and a second nucleotide sequence, wherein said first nucleotide sequence is homologous to the nucleotide sequence of the first homology arm on said vector DNA strand, and said second nucleotide sequence is designed to be homologous to the nucleotide sequence of a first terminus on a target DNA strand; and

c) a second double-stranded adaptor oligonucleotide comprising a second oligonucleotide strand comprising, in the following order, from 3' to 5': a third nucleotide sequence and a fourth nucleotide sequence, wherein said third nucleotide sequence is homologous to the nucleotide sequence of the second homology arm on said vector DNA strand and said fourth sequence is designed to be homologous to the nucleotide sequence of a second terminus on said target DNA strand.

32. The kit of Claim 25, 28, or 31 wherein the DNA vector is purified.

33. The kit of Claim 28 or 31 wherein the DNA vector, the first double-stranded oligonucleotide, and the second double-stranded oligonucleotide are purified.

34. The kit of Claims 25, 28, or 31 wherein the target DNA molecule comprises bacterial, viral, parasite, or protozoan DNA.

35. The kit of Claims 25, 28, or 31 wherein the target DNA molecule comprises a genetic mutation or polymorphism known or suspected to be associated with a disorder or disease.

36. The kit of any one of Claims 25-29 wherein the bacterial recombinase is RecE/T or Redα/β recombinase or both RecE/T and Redα/β recombinases.

37. The method of any one of Claims 2-11 wherein the target DNA is known or suspected to be associated with a disorder or disease when genetically mutated.

38. The method of any one of Claims 2-11 wherein the target DNA is a bacterial, viral, parasite, or protozoan DNA.

39. The method of Claims 2, 4, 6, 8, or 10 which further comprises detecting a recombinant DNA molecule that comprises the target DNA inserted into the vector.

40. The method of Claims 3, 5, 7, 9, or 11 which further comprises detecting a recombinant DNA molecule that comprises the target DNA inserted into the vector.

41. A method of detecting the presence of an infectious agent comprising carrying out the method of Claim 39, wherein the target DNA is derived from a patient suspected of having the infectious disease, and the sequences of the first and second homology arms are homologous to the sequences present in DNA of the infectious agent.

42. A method of detecting the presence of an infectious agent comprising carrying out the method of Claim 40, wherein the target DNA is derived from a patient suspected of having the infectious disease, and said second and fourth nucleotide sequences are homologous to sequences present in DNA of the infectious agent.

43. The method of Claim 41 or 42 wherein the infectious agent is a virus, bacteria, protozoa, fungus, or parasite.

44. A method of detecting the presence of a genetic condition, disease, disorder, or polymorphic trait comprising carrying out the method of Claim 39, wherein the target DNA is derived from a patient suspected of having a genetic condition, disease, disorder, or polymorphic trait, and the sequence of the first homology arm is homologous to the sequence upstream from a site known or suspected to be associated with the genetic condition, disease, disorder, or polymorphic trait, and the sequence of the second homology arm is homologous to the sequence downstream from a site known or suspected to be associated with the genetic condition, disease, disorder, or polymorphic trait.

45. A method of detecting the presence of a genetic condition, genetic disease, genetic disorder, or polymorphic trait comprising carrying out the method of Claim 40, wherein the target DNA is derived from a patient suspected of having the genetic condition, genetic disease, genetic disorder, or polymorphic trait, and the sequence of the first double-stranded oligonucleotide is homologous to the sequence upstream from a site known or suspected to be associated with the genetic condition, genetic disease, genetic disorder, or polymorphic trait, and the sequence of the second double-stranded oligonucleotide is homologous to the sequence downstream from a site known or suspected to be associated with the genetic condition, genetic disease, genetic disorder, or polymorphic trait.

46. The method of Claims 44 or 45 wherein the genetic condition, genetic disease, genetic disorder, or polymorphic trait is or predisposes the patient to cancer, asthma, arthritis, drug resistance, drug toxicity, or a neural, neuropsychiatric, metabolic, muscular, cardiovascular, or skin condition, disease or disorder.

47. The vector of Claim 19 wherein the vector further comprises a selectable marker located outside the first and second homology arms, such that the vector comprises, in either of the following two orders from 5' to 3' along a vector DNA strand: i) the first homology arm, the selectable marker, the origin of replication and the second homology arm, or ii) the first homology arm, the origin of replication, the selectable marker, and the second homology arm.

48. The vector of Claim 47, wherein the sequence of the vector does not contain one or more direct repeats of a sequence of at least five or more nucleotide base pairs either 5' or 3' to both the origin of replication and the selectable marker.